Liquid Chromatography Problem Solving and Troubleshooting

Question:

I observe an increase in pressure during a reversed-phase gradient analysis of modified protein(s). I assume that something is precipitating out on my column or on my frit and redissolving later. I am concerned that this is interfering with or biasing my analysis. Is there a way to clean up my sample prior to analysis so that I don't have this pressure problem?

Answer:

First let me say that I believe your "problem" is not a problem and that your hypothesis about the observed behavior is incorrect. A more plausible explanation of the described observation is that you are observing normal behavior in HPLC; the back pressure is proportional to the viscosity of the mobile phase, and the viscocity will change during the gradient. You must test for this possible explanation before you proceed to hypothesize that a precipitate is causing the pressure increase.

To illustrate the viscosity behavior, refer to Figures 1 and 2. Figure 1 is a plot of viscosity versus percent organic solvent of several different commonly used reversed-phase solvents. Note that mobile phases consisting of mixtures of organic solvent and water have higher viscosity near the middle of their mixture ratio than on either end (100% water or 100% organic solvent). Figure 2 is an actual pressure profile of an HPLC blank gradient run with no sample injected, one for water to methanol and one for water to acetonitrile. By comparing these two figures, it can be seen that the pressure profile of the gradient is quite similar to the viscosity versus percent organic solvent relationship in Figure 1.

To elucidate your situation, I suggest that you run a blank gradient and record the rise in pressure (the pressure profile) that occurs during the gradient run. Once that experiment is completed, compare that pressure profile with the one obtained when you injected the sample. If the two pressure profiles are similar, precipitating of sample material is not a problem. In other words, things are "chromatographically normal." If there is a significant difference in the two profiles, drop me a note and I will discuss that situation in another article.

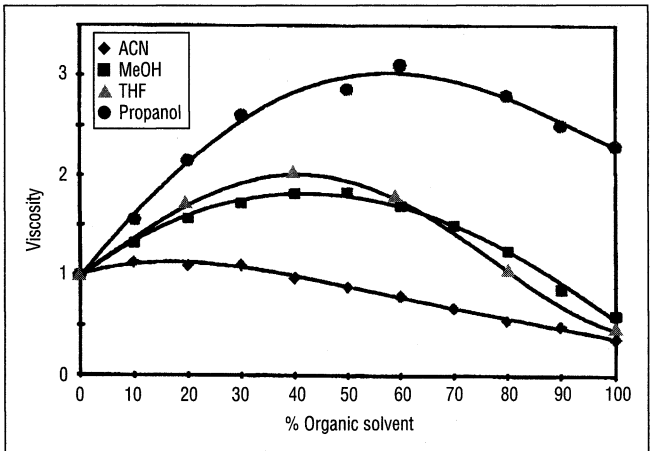

Figure 1. Viscosity as a function of content of aqueous mobile phase.

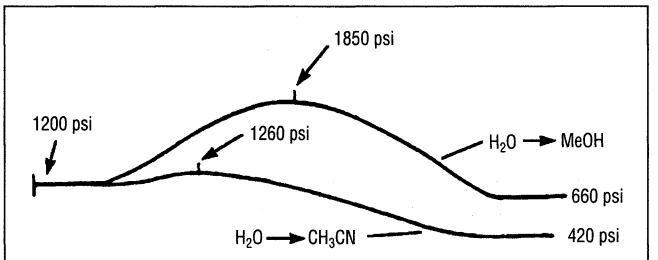

Figure 2. Gradient pressure output (Reprinted from B.A. Bidlingmeyer, Practical HPLC Methodology and Applications, J. Wiley & Sons, New York, 1993).

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

Brian A. Bidlingmeyer
Associate Editor